Aktivering van het vruchtbeginsel van Petunia

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AKTIVERING VAN HET VRUCHTBEGINSEL VAN PETUNIA

PROMOTOR

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AKTIVERING VAN HET VRUCHTBEGINSEL VAN PETUNIA

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Aan allen die op enigerwijze hebben bijgedragen tot dit proefschrift

Inleiding

Bij de geslachtelijke voortplanting van de bloemplanten kan men 3 fasen onderscheiden, welke achtereenvolgens doorlopen worden, en leiden tot de uiteindelijke bevruchting en embryo-ontwikkeling (Linskens 1973):

1. de bestuiving, het brengen van de pollenkorrel met generatieve cel op de stempel.
2. de progame fase, waarin alle processen plaats vinden, die leiden tot het verplaatst worden van de mannelijke geslachtscellen via de pollenbuizen in de stempel en de stijl naar het vruchtbeginsel. Deze fase wordt gekenmerkt door een zeer intensieve wisselwerking tussen de groeiende pollenbuis enerzijds en het geleidingsweefsel van de stijl anderzijds. Met het ledigen van de pollenbuis in de synergide van de embryozak eindigt de progame fase en begint de
3. gamogene of bevruchtingsfase. Hierin vindt de versmelting plaats van de mannelijke en vrouwelijke geslachtscel en van de mannelijke geslachtscel met de secundaire embryozakcel.

Reeds vroeg in de geschiedenis van het bevruchtingsonderzoek van de bloemplanten wordt in de literatuur op post-florale processen gewezen, die zich tijdens de progame fase afspelen in andere bloemdelen dan de stijl. Deze processen schenen geïnitieerd en gestuurd te worden door de interactie van de pollenbuis en de stempel en de stijl. Fitting (1909) onderscheidde hierin de volgende stadia, die gelijktijdig met de progame en gametogene fase in de bloemen van tropische orchideeën verlopen:

a. het verleppen van de bloembladeren,
b. het dichtklappen van de lobben van de stempel en het opzwellen van het gynostemium,
c. het opzwellen van het vruchtbeginsel,
d. het groen worden van de perianth.

Deze post-florale processen konden ook door andere dan normale bestuivingen geïnduceerd worden. Het sluiten van het stigma en het opzwellen van het gynostemium werden door Fitting, ook na toedienen van een waterig extract van pollinïen op het stigma, waargenomen. Hij analyseerde ook de stoffen, die voor de inductie van deze post-florale processen verantwoordelijk waren, nader op enkele chemische eigenschappen (Fitting 1910).

Deze stoffen werden door hem " Reizstoffe " genoemd. Hij stelde voor, in navolging van waarnemingen over dergelijke stoffen in zoologische objecten, ook deze stoffen hormonen te noemen.
Ook Hsiang (1951) vond binnen 48 uur na bestuiving bij Cymbidium verwelking van de perianth, lokale opzwelling en vergroting van de columna en verkleuring van de columna door de vorming van anthocyaan, voordat er een bevruchting had plaatsgevonden. Ook kon hij een verlies in drooggewicht van de perianth en toename in drooggewicht van de columna aantonen. De aktivering van het vruchtbeginsel in het bijzonder de zaadvorming zonder bevruchting, agamospermie, is door Tschermak-Seyenegg (1949) beschreven.

Veel interessanter zijn echter aktiveringsprocessen in de vruchtbeginsels na bestuiving, echter vóórdat de pollenbuizen het vruchtbeginsel hebben kunnen bereiken. Millardet beschreef in 1901 de vorming van vruchten zonder zaad na bestuivingen van Vitis vinifera met pollen van Ampelopsis. Massart (1902) vond na zelf-inkompatibele bestuivingen van meloenen en pompoenen een ontwikkeling van holle vruchten zonder zaadontwikkeling. Ook Yasuda (1934 a en b, 1939) meldde het ontstaan van vruchten na bestuiving zonder dat er een bevruchting plaats kon hebben gevonden bij Petunia.
Bij enkele Citrus soorten vermeldden Nagai en Tanikawa (1926) de vorming van zaadloze vruchten na zelf-bestuiving en niet na kruisbestuiving. Erwin en Haber (1929) voerden kruisingen uit tussen enkele Cucurbita soorten waarna vele zaadloze vruchten gevormd werden. In zijn overzichtsartikel van 1942 gaf Gustafson een uitgebreide opsomming van soorten waar zaadloze vruchten ontstonden langs kunstmatige of natuurlijke weg. Ook bij Orchideeën bloemen komen er ontwikkelingen in het vruchtbeginsel op gang na bestuiving maar nog voor de bevruchting heeft plaats gevonden. Gessner (1948) en Oertli en Kohl (1960) konden aantonen, dat er na bestuiving een verhoogd transport van eiwitten, energierijke stoffen en water plaats vond naar het vruchtbeginsel toe. Knauft et al. (1970) vond na bestuiving van Cymbidium een verhoogde peroxidase activiteit en zetmeelophoping in het vruchtbeginsel.

Ook trad er anthocyaan vorming op waarbij kon worden aangetoond dat de hiervoor benodigde enzymen nieuw gevormd waren. Duncan en Curtis (1942) vonden zelfs een stimulatie van de vruchtontwikkeling bij sommige tropische orchideeën nog voor de polliniën gekiemd waren. Aktiveringsprocessen van het vruchtbeginsel na bestuiving maar voor het tot stand komen van de bevruchting konden met moderne methoden ook voor Petunia aangetoond worden (Linskens 1973).

Uit bovenstaande gegevens kan gekonkludeerd worden, dat het vruchtbeginsel tijdens de progame fase niet een toestand van passief afwachten doormaakt, maar blijkbaar zich reeds voorbereid op wat er gaat komen in de gamogene fase.

In de volgende studies is getracht meer licht te werpen op veranderingen in de biochemische aktiviteit van het vruchtbeginsel van Petunia tijdens de progame fase, vooral in verband met inkompatibele bestuivingen. Bij dit type van bestuivingen komt het bij Petunia weliswaar nog tot een eerste kontakt tussen de pollenkorrel of pollenbuis en het vrouwelijk weefsel (stigma en stijl) maar de gamogene fase wordt niet bereikt.. De vraag kan dus gesteld worden of een aktivering van het vruchtbeginsel, zoals dat zich na een kompatibele bestuiving manifesteert, zich ook na een inkompatibele bestuiving voordoet.

Deze vraag is in de volgende detail problemen te verdelen:

1. Veranderingen in de intensiteit van het eiwit-metabolisme:
   a. Zijn er kwantitatieve veranderingen in de hoeveelheid eiwitsynthese aan te tonen welke specifiek zijn voor de aard van de bestuiving, kompatibel of inkompatibel?
   b. Welke veranderingen in de eiwit-synthese van vruchtbeginsels van onbestoven bloemen kunnen worden aangetoond tijdens de verwelking?
2. Zijn er naast de verschillen in de intensiteit van de eiwit-synthese tussen bestoven en onbestoven, kruis-en zelfbestoven vruchtbeginsels ook specifieke verschillen aantoonbaar in de proteine-samenstelling?

3. Zijn de veranderingen in het eiwit-metabolisme van de vruchtbeginsels in de progame fase afhankelijk van de genetische samenstelling van het pollen en/of het vruchtbeginsel bij gelijkblijvende inkompatibiliteits-allelen?
4. Kan er iets gezegd worden over de stof welke de informatie over de aard van de bestuiving overdraagt van het stigma of de stijl naar het vruchtbeginsel?

Aangezien het Botanisch Laboratorium Nijmegen beschikt over een grote kollektie klonen van de zelf-inkompatibele Petunia hybrida konden de hierboven aangegeven vraagstellingen als volgt ter hand worden genomen:

1. Uit de vruchtbeginsels van een heterologe kloon werden op meerdere tijdstippen na bestuiving, maar vóórdat de gamogene fase begon, de polysomen geëxtraheerd. De hoeveelheid polysomaal materiaal en de eiwit-synthese aktiviteit worden in een in vitro systeem gemeten.
2. De hoeveelheden extraheerbaar polysomaal materiaal en de aktiviteit in het in vitro systeem werden ook gemeten bij vruchtbeginsels van een, voor de inkompatibiliteits-allelen homologe kloon.
3. De veranderde eiwit-synthese capaciteit van de polysomen, die tijdens de progame fase uit de vruchtbeginsels waren geïsoleerd, werd nader bestudeerd met behulp van de transcriptie van het m-RNA uit geëxtraheerde polysomen in een in vivo systeem (onbevruchte eieren van Xenopus laevis). De in dit systeem afgelezen eiwitten werden verder onderzocht door middel van gel-elektroforese.
4. Uitgaande van de hypothese dat bij de overdracht van informatie van het stigma of de stijl naar het vruchtbeginsel een chemische stof betrokken zou zijn, werden orienterende proeven verricht naar de aard van deze stof met behulp van radioaktief gemarkeerde pollen.

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Planta

In vitro Protein Synthesis with Polysomes from Unpollinated, Cross- and Self-pollinated *Petunia* Ovaries

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Summary. From *in-vitro* protein synthesis studies it has been shown that in *Petunia hybrida* (clone W166K, incompatibility genes S_1S_2) the ovary is stimulated to synthesize proteins before the pollentubes reach the ovary A difference in protein metabolism is detected after self- and cross-pollination On the basis of this result has been concluded that a signal has to be sent from the stigma or the style towards the ovary which induces the changes in metabolic activity The signal must be different for cross- and self-pollination

Introduction

The processes, which lead to the fertilization of the ovule, can be divided into three stages· pollination, the programic phase and the syngamie of the reproductive cells

During the programic phase, an intensive metabolic interaction exists between the growing pollentube and the conductive tissue of the style Also organs, which have no direct contact with the pollentube may react to the presence of the pollentube in the style In orchid flowers great metabolic changes occur after pollination and the production of odorous compounds is stopped (Hsun and Hsiang, 1951, Knauft *et al*, 1970) Roggen (1967) found an activity wave of some enzymes in the style in front of the growing pollentube Dramatic changes in the relative content of free aminoacids, total RNA and total protein of the ovaries were observed in a period between pollination and fertilization (Linskens, 1973) Translocation studies have shown that the metabolic state of all parts of the flower is different after pollination and before fertilization (Linskens, 1974) In this paper we looked for changes in the protein metabolism in ovaries of *Petunia* after self- and cross-pollination For this purpose, an *in-vitro* system was choosen in which polysomes from ovaries were able to synthesize protein with the aid of enzymes from a rat liver supernatant.

Methods and Material

Buds of *Petunia hybrida* clone W166K (with incompatibility genes S_1S_2) with a length of about 5 6 cm (this is about 24 h before anthesis) were emasculated and 24 hours later pollinated with W166K pollen (self-pollination) or T_2U pollen (cross-pollination) Unpollinated flowers served as a control At different times after pollination flowers were cut and the ovaries stored in liquid nitrogen until extraction of the polysomes

45 frozen ovaries were ground in a mortar cooled with liquid nitrogen and the powder dissolved in 9 ml of extraction medium containing 50 mM Tris/HCl pH = 8 3 17 5 mM Mg acetate 175 mM NH_4Cl 350 mM sucrose, 4 mg/ml RNA and 5 mM mercapto ethanol Sodium deoxycholate (DOC) was added to give a final concentration of 7 mM (Breen *et al* 1972) All handlings were carried out at 0° C

After standing for at least 10 minutes, the suspension was centrifuged for 10 min at 20 000 × *g* in a Sorvall centrifuge The supernatant was carefully layered on a discontinuous sucrose gradient consisting of 2 ml 2 M sucrose and 1 ml 1 M sucrose in medium B, which contained 10 mM Tris/HCl pH 8 3, 10 mM NH_4Cl 5 mM Mg acetate, 5 mM mercapto-ethanol

After centrifugation for 90 min at 368 000 × *g* (Spinco L2 65k 65 000 rpm) or 70 min at 503,000 × *g* (Spinco L2 75 75 000 rpm) the pellet was suspended in 0 45 ml of medium B

The suspension of polysomes was used for three purposes (a) protein synthesis in a cell-free system, (b) determination of the polysomal protein content, and (c) sucrose density-gradient centrifugation

Cell free Protein Synthesizing System
(Linskens *et al* 1970)

The incubation mixture was composed of 45 µmol Tris/HCl pH = 7 6 38 µmol NH_4Cl 3 8 µmol Mg acetate 10 µmol KCl 6 µmol pyruvate kinase, 2 µmol phosphoenol pyruvate 0 75 µmol ATP 0 10 µmol GTP, 15 µmol of each of the 19 I -amino acids 15 µmol ^{14}C leucine (0 87 µCi) 20 µl liver supernatant (Konings *et al* 1969) 2 9 µmol mercapto-ethanol and polysomes (50 100 µg

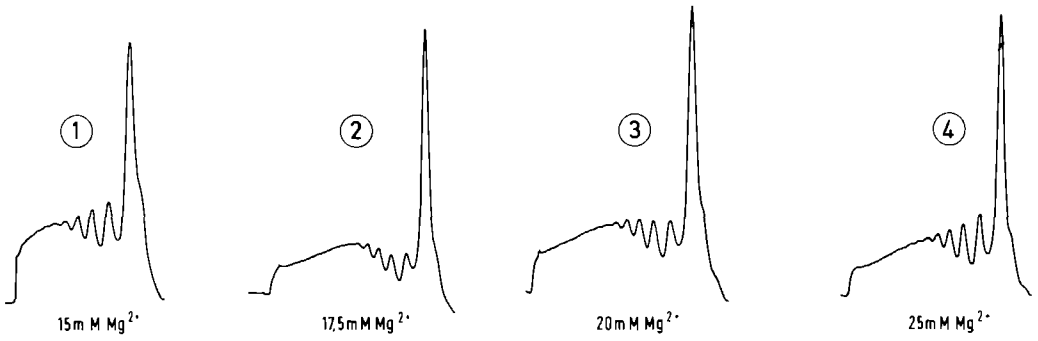

Fig. 1. Polysomal profiles on sucrose density-gradient after extraction of the polysomes with several Mg^{2+} concentrations

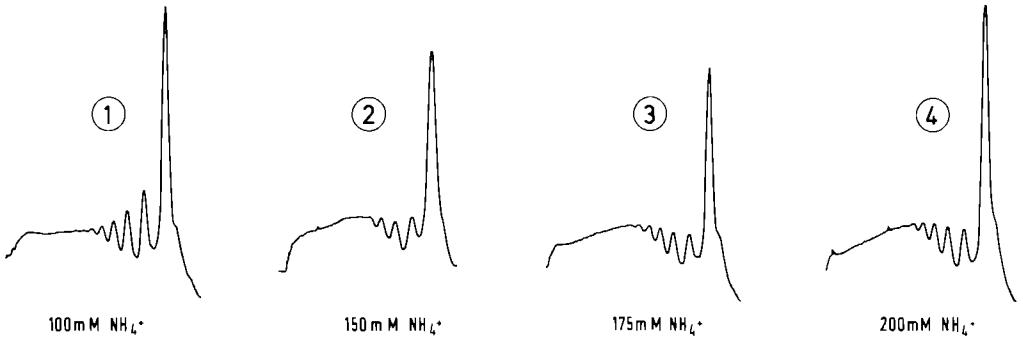

Fig. 2. Polysomal profiles on sucrose density-gradient after extraction with several NH_4^+ concentrations

polysomal protein) The total volume was 610 µl After incubation for 30 min at 37° C the reaction was stopped by addition of 2 ml ice-cold 0 6 M TCA Further handlings were according to Linskens *et al* (1970)

Quantitative Determination of Polysomal Protein

To 50 µl solution of polysomes, 50 µl medium B and 100 µl 2 M NaOH was added After hydrolysis for 1 h at 37° C (Bloemendal *et al*, 1964) the content of polysomal protein was determined according to Lowry *et al* (1951)

Sucrose Density-gradient Centrifugation

50–100 µl polysomes solution was layered on a linear sucrose gradient (0 6–1 2 M in medium B) and centrifuged at 300,000 × *g* for 35 min (Spinco L2 75, SW 50-I, 50,000 rpm) The bottom of the centrifuge tube was punctured and the absorption measured at 254 nm

Chemicals

ATP (Adenosin-5'-triphosphate di-Na-salt), GTP (Guanosin-5'-triphosphate tri-Li-salt) and pyruvate kinase (from rabbit muscle) were purchased from Boehringer and Sohne GmbH Mannheim. Phosphoenol pyruvic acid tri-Na salt, RNA (Ribonucleic acid from Torula yeast grade VI) and the L-amino acids were obtained from Sigma Chemicals L-[^{14}C]leucine (spec act 58 mCi/mmol) was from the Radiochemical Centre, Amersham

Results

1. Pollentube Growth

The growth of the pollentubes showed the following pattern (Linskens and Esser, 1959): the pollentubes from a self- and a cross-pollination grew through the style with about the same speed up to 24 h after pollination. Thereafter, the tubes at self-pollination decreased in growth rate, whereas at cross-pollination the tubes continued their growth at the same rate. The "cross-pollentubes" reached the ovary at about 50 h after pollination, whereas the "self-pollentubes" did not reach the ovary even at about 70 h after pollination, the moment where the withering of the style had already started.

2. Adaptation of the Extraction Medium

To adapt the extraction medium (Linskens *et al.*, 1970) to the green plant material of Petunia, the concentrations of NH_4^+ and Mg^{2+} were varied to establish the optimal concentrations. The results are presented in Figs. 1 and 2 respectively.

Preliminary experiments had shown that less degradation of the polysomal material occurred at about 150 mM NH_4^+, when the other compounds in

the extraction medium were kept at concentrations according to Linskens *et al* (1970) On the basis of this result the exact optimum for Mg^{2+} was determined at 17 5 mM Mg^{2+} The results are shown in Fig 1

Subsequently the optimum concentration of NH_4^+ was again determined at 175 mM (results shown in Fig 2) Optimum results are considered to be obtained at concentrations which cause little degradation of the polysomes, so, with the greatest hexamer/dimer ratio (Breen *et al*, 1972)

Increasing the pH of the extraction medium had no significant effects, however, a slight increase in hexamer/dimer ratio was obtained at pH = 8 3

3 *In-vitro Protein Synthesis*

Linskens *et al* (1970) have shown that, besides the content of liversupernatant, also the Mg^{2+} concentration in the incubation mixture greatly influenced the incorporation of [^{14}C]leucine Therefore, the optimum concentration of Mg^{2+} was determined using 20 µl liversupernatant Fig 3 shows the incorporation in relation to Mg^{2+} concentration Optimum value is 3 8 µmol Mg^{2+}

From table 1 can be read that highest incorporation of [^{14}C]leucine was achieved using 20 µl of our liversupernatant This contained 750 µg protein (Lowry *et al*, 1951)

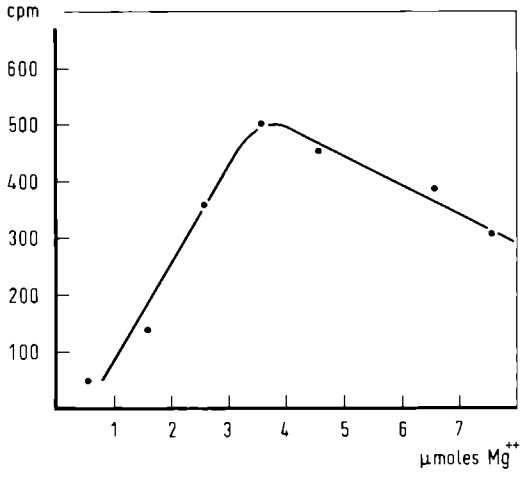

Fig. 3. Incorporation activity of [^{14}C]leucine in relation to the content of Mg^{2+} in the incubation mixture

Table 1. Incorporation activity of [^{14}C]leucine in dependence of the content of rat liver supernatant in the incubation mixture

µl liver supernatant	Counts per min
5	296
10	386
20	662
30	645
40	601

4 *Ribosomal Protein in Ovaries of Petunia after Pollination*

The relative changes in ribosomal protein of ovaries from cross-, self- and unpollinated flowers are shown in Fig 4 The content of ribosomal protein of the ovaries at the moment of pollination is set down as 100% The absolute amount of ribosomal protein is about 6–7 µg per ovary In the first hours after pollination no great differences are detectable in either

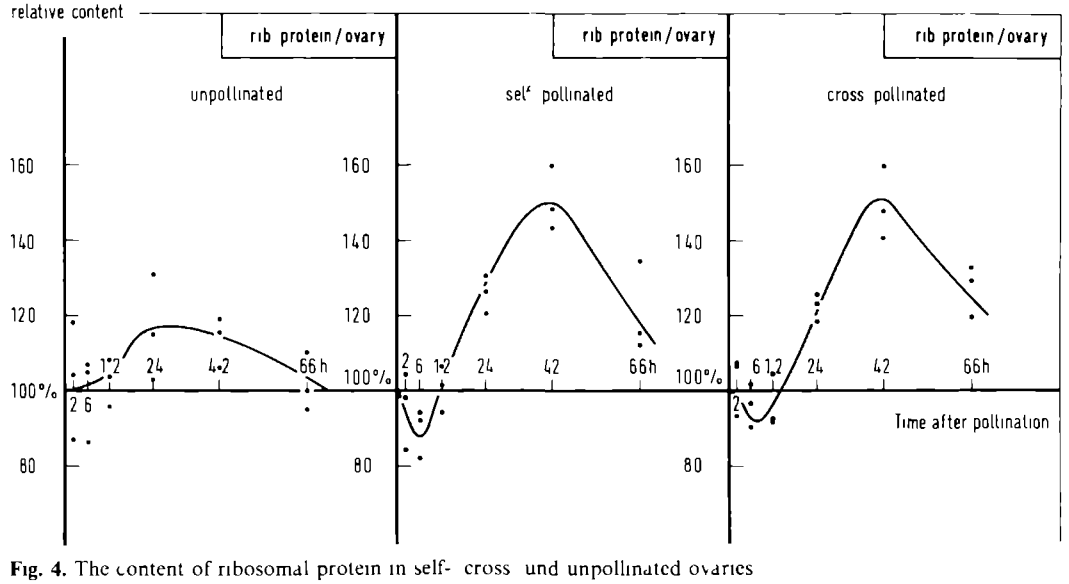

Fig. 4. The content of ribosomal protein in self- cross und unpollinated ovaries

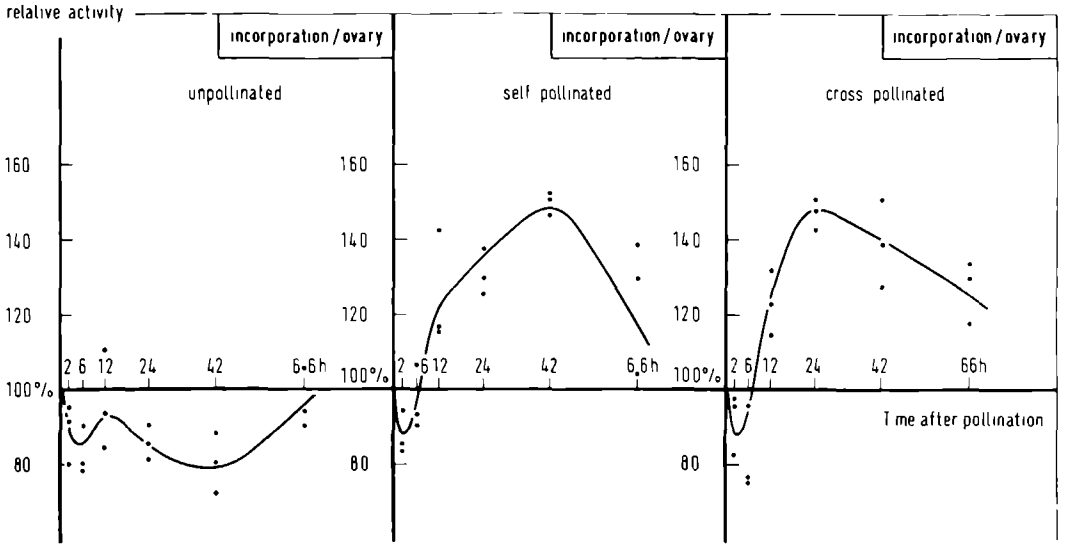

Fig 5 Incorporation activity of polysomes from self cross and unpollinated ovaries

cross- or self-pollinated and unpollinated ovaries A minimum amount was found at 6 h after pollination From 6 to 24 h after pollination all ovaries showed an increase in ribosomal protein content Until 24 h there were no significant differences between the ribosomal content of pollinated and unpollinated ovaries, or between cross- and self-pollinated ones The pollinated ovaries showed a further increase in the content of ribosomal protein between 24 and 42 h after pollination, whereas the unpollinated ovaries showed some decrease at 42 h This decline continued to 66 h after pollination The pollinated ovaries reached their maximum ribosomal protein content at 42 h and a subsequent decrease to 66 h At no point of time after pollination a significant difference was observed between self- and cross-pollinated ovaries with regard to the ribosomal protein content In contrast, however, significant differences were obtained between pollinated and unpollinated ovaries at 42 h after pollination and thereafter

5 Incorporation of [^{14}C]leucine

The incorporation per ovary, expressed as percentage of the incorporation of [^{14}C]leucine by polysomes from ovaries extracted at the moment of pollination, is shown in Fig 5 There is a decrease in incorporation activity up till about 2 6 h after pollination

Then, in all three cases (cross- self- and unpollinated) an increase in incorporation activity took place The maximum for unpollinated ovaries seems to be reached at about 12 h The self- and cross-pollinated ovaries showed a stronger increase at 12 h after pollination than the unpollinated ones Until this moment the cross- and self-pollinated ovaries did not differ significantly from unpollinated ovaries From 12 h onwards a clear differences in the incorporation activity between pollinated and unpollinated ovaries is observed

The pollinated ovaries showed a further increase subsequent to 12 h after pollination, whereas the activity in the unpollinated ones decreased The cross-pollinated ovaries reached their maximum of incorporation at about 24 h The self-pollinated ones, however, continued their (slower) increase until 42 h after pollination Thereafter a decrease could be seen in the incorporation activity of both cross- and self-pollinated ovaries up to 66 h

After 42 h the unpollinated ovaries showed again an increase up to 66 h

Discussion

The hexamer/dimer ratio is used as a parameter for the determination of the optimal concentrations of Mg^{2} and NH_4^{+} Figs 1 and 2 show that higher ionic strength results in better ratios, i e better extraction conditions These results are in good agreement with those of Breen *et al* (1972) and Davies *et al* (1972) As can be seen from Figs 4 and 5 the unpollinated ovaries are metabolically less active and only certain processes proceed Fig 4 demonstrates a stimulation of protein synthesis in the unpollinated ovary at 66 h following an earlier decrease In order to explain this protein synthesis one could speculate that hydrolytical enzymes are synthesized, necessary for the withering process which starts at about 70 h

A difference in reaction after pollination can be detected as early as 6 12 h after pollination From Fig 5 is concluded that after about 6 h after pollina-

tion the incorporation activity of the polysomes extracted from pollinated ovaries is higher than from unpollinated ones. A difference between pollinated and unpollinated ovaries in the ribosomal content is not observed before 24 h after pollination

Differences in metabolic activities between cross- and self-pollinated ovaries become apparent at about 12 h after pollination In all experiments, maximum incorporation took place at 24 h in cross-pollinated ovaries, whereas in self-pollinated ovaries maximum incorporation occurred at 42 h (Fig. 5). Since the pollentubes did not reach the ovary before 50 h after pollination, we have to conclude that the metabolic activity of the ovaries had changed before pollentubes entered the ovary A signal (or signals) must have been sent from the stigma or the style to the ovary to induce the change in metabolic activity.

Since cross-pollinated ovaries showed a different metabolic activity in respect to self-pollinated ones (Fig. 5) the above mentioned signal must also include information about the kind of pollen on the stigma. Until the moment, the signal arrives, all ovaries follow the same profile of metabolism.

These results are in agreement with those of Linskens (1973) concerning the content of ribosomal RNA/ovary, the total content of protein and amino-acids. Exception must be made for the time scale, which could be due to different growing-conditions of the plant material.

About the nature of the signal can only be speculated. Sinyukhin and Britikov (1967) have found a signal of electrical nature, which occurs in the style after pollination. In our laboratory, experiments have been carried out with *Petunia*, which also indicate the existence of electrical potentials in the style after pollination (Linskens and Spanjers, 1973).

The function of the stimulation of the ovary is unclear. It could be, that it conditions the tissues in the ovary, allowing optimal development of the eventually formed embryo.

Experiments are in progress with regard to possible qualitative differences in the proteins formed at several periods after pollination Perhaps these will deliver a clue to the possible function of the early stimulation of the ovary after pollination.

Although the absolute values varied from experiment to experiment, the trend i.e remained always the same. These differences are probably due to the varying growing-conditions in the greenhouse with regard to temperature, water supply etc. For instance the temperature measured at sunny days rose to 35° C, whereas on cloudy and cool days, the temperature reached only 16–18° C. From Vallade and Cornu (1973) it is known that the moment of fertilization in Petunia is very dependent on the temperature at which the plants are cultivated. They found that at 20° C the fertilization took place 56 h after pollination, whereas at 25° C this process occurred at 40 h after pollination. As far as the incompatibility reaction is concerned, it can be mentioned, that in each experiment some flowers were not cut from the plants. At about 2 weeks after pollination the ovaries were examined, and it was always found that cross-pollinated flowers had thickened ovaries with many (unripe) seeds, whereas the unpollinated and self-pollinated flowers had shrunken and dried ovaries. These results confirmed that the incompatibility reaction was fully intact.

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ACTIVATION OF PROTEIN SYNTHESIS IN OVARIES FROM PETUNIA HYBRIDA AFTER COMPATIBLE AND INCOMPATIBLE POLLINATION

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SUMMARY

Differences in protein metabolism after cross- or self pollination are found in Petunia ovaries, before pollentubes reach the ovary Similarities and differences with another clone from *Petunia hybrida* are discussed, as well as possible functions of the mentioned phenomena

1 INTRODUCTION

Several authors already described changes in the metabolism after pollination in flower parts which were not in direct contact with the growing pollentubes (HSUNG & HSIANG 1951, KNAUIT et al 1970, ROGGIN 1967, HALL & FORSYTH 1967) LINSKENS (1973) mentioned changes in the RNA, amino acid and protein content in the ovaries after pollination He also found an altered transport of sugars and amino acids in all parts of the *Petunia* flower after cross- or self-pollination (1974)

In a previous report (DIJKENBERG 1976) changes in protein metabolism in the ovaries after pollination which occurred *before* the pollentubes reached the ovary, have been described whereas also differences in reactivity of the ovary after cross- and self-pollination were observed In order to establish whether this phenomenon, described for *Petunia hybrida*, clone W166K was restricted to this special clone or was a more general phenomenon, similar experiments were carried out with clone T2U using the same incompatibility alleles but in a different genetic background

2 METHODS AND MATERIAL

Buds of *Petunia hybrida* clone T2U, incompatibility alleles S_1S_3 were emasculated 24 hours before anthesis One day later the flowers were pollinated with T2U pollen (selfed) or with W166K pollen (incompatibility alleles S_1S_2, crossed) A third part of the flowers was left unpollinated as a control

At different times after pollination, the flowers were cut and the ovaries stored in liquid nitrogen Polysomes were extracted The content of ribosomal protein was determined and the incorporation capacity of ^{14}C-leucine was measured in an in vitro system containing soluble enzymes and co-factors from rat livers

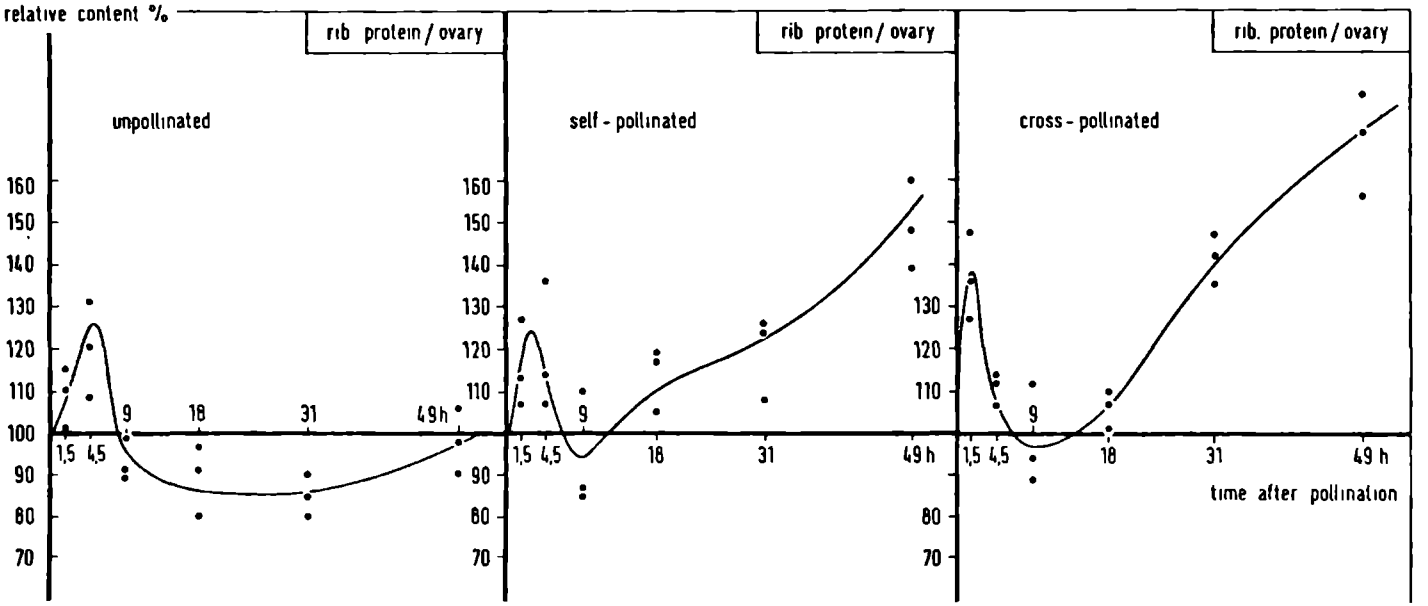

Fig 1 Relative ribosomal content per ovary in self-, cross- and unpollinated ovaries

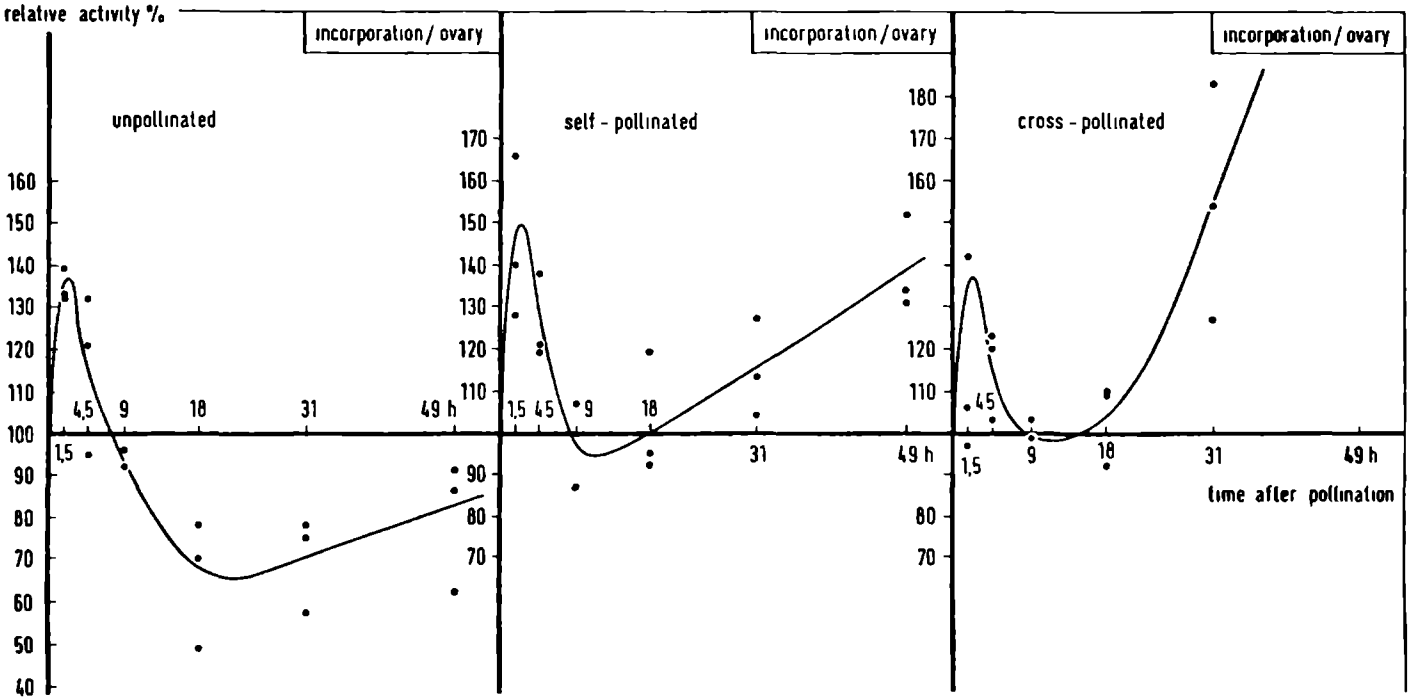

Fig 2 The relative incorporation activity per ovary in self-, cross- and unpollinated ovaries

All methods and materials were as described in a previous publication (DEURENBERG 1976). Plants were grown in the greenhouse at 20–25 °C during daytime and 15–18 °C during the night.

RESULTS

3.1 Pollentube growth

After cross-pollination the pollentubes grow through the style into the ovary in about 30 hours under greenhouse conditions. In contrast to that, after selfpollination the pollentubes grow much slower and do not reach the ovary even after 60 hours.

As an additional control on the incompatibility-reaction, some flowers were not cut from the plant. At about two weeks after pollination, the ovaries from cross-pollinated flowers contained many (unripe) seeds. The ovaries after self-pollination, however, were shrunken and dried and contained no or only a few (unripe) seeds.

3.2 Ribosomal proteins content

Fig. 1 shows the content of ribosomal proteins of the ovaries after cross- and self-pollination and of unpollinated flowers. The content of ribosomal proteins at the moment of pollination is set down as 100 %. At that moment, the amount of ribosomal proteins was about 8 μg per ovary, as determined according to BLOEMENDAL et al. 1964 and LOWRY et al. 1951).

In the first hours after pollination, until about 9 hours, there were no differences in the amount of ribosomal proteins between pollinated (crossed or selfed) and unpollinated ovaries. In all three cases a first maximum was found between 1.5 and 4.5 hours after pollination. Subsequently the protein content declined until about 9 hours.

Whereas in the unpollinated ovaries the amount of ribosomal proteins decreased farther till 20 hours, an increase was found in the pollinated ovaries from 9 hours after pollination and on. After cross-pollination the increase was greater than after self-pollination.

3.3 Incorporation activity of ^{14}C-leucine

As shown in *fig. 2*, an increase in incorporation capacity was found with polysomes from pollinated as well as from unpollinated ovaries. This increase reached a first maximum between 1.5 and 4.5 hours after pollination. Then, a decrease occurred, which showed a minimum at about 20 hours with polysomes from unpollinated ovaries. However, after pollination, crossed or selfed, the minimum was reached at about 10–12 hours. With polysomes from unpollinated ovaries a slight increase was found from 18 till 49 hours. After pollination, the increase after about 12 hours was a very strong one, especially after cross-pollination.

Between 18 and 30 hours after pollination, a difference in incorporation capacity appeared with polysomes from cross-pollinated flowers, and with those after self-pollination. Whereas the incorporation activity at 31 hours

after self-pollination was about 120% polysomes from cross-pollinated ovaries showed an increase of the incorporation activity of about 150–160%. At 49 hours, the incorporation activity with polysomes after cross-pollination was about 250–300%, whereas after self-pollination this amounted to 140%.

4 DISCUSSION

As *fig. 1* shows, a difference in reaction of the ovary from pollinated and unpollinated flowers appeared not earlier than about 9 hours after pollination. After cross- or self-pollination, a sudden increase was observed in the ribosomal protein content, whereas in the untreated flowers the ribosomal protein content in the ovaries remained fairly constantly between 9 and 31 hours.

The incorporation activity increased after 9 hours after pollination, crossed or selfed, whereas in unpollinated flowers the incorporation decreased farther. Since the pollentubes only grow into the style for about 1/3 of the style length at 9 hours after pollination, some kind of signal has to be derived from the stigma or the style. This signal translocates to the ovary in order to induce the activation of the protein metabolism.

From *fig. 2* it is clear, that there does not only exist a difference in reaction between pollinated and unpollinated ovaries, but also between cross- and self-pollinated ones. The increase in incorporation activity after cross-pollination is much stronger than after self-pollination. This greater increase has been found as early as between 18 and 31 hours and also was found in a later stage. This means, that the ovary differently reacts after self- or cross-pollination at a moment that no pollentube could have reached the ovary. So, is seems justified to conclude that the above mentioned signal also contained information about what kind of pollination has taken place.

Whether there occur two signals, one having information about pollination or not, and another about the *kind* of pollination, selfed or crossed, one only can speculate. The results here presented are in good agreement with those described in a previous paper (DEURENBERG 1976), where similar experiments were carried out with clone W166K. So, the above mentioned phenomena of reactivity of the protein metabolism of the ovary before a direct contact of the pollentubes with the ovary seem to be a general one in Petunia.

The fact, that recognition of pollination and possibly also the kind of pollination has occurred before 9 hours after pollination, is in agreement with results mentioned by VAN DER DONK (1974). He established the recognition of cross- or self-pollination in a very early stage of the pollentube/style interaction. GIESSEN (in the press) showed, that changes in the wilting processes of the flower after pollination only can take place in case pollen has germinated and grown into the style. About 3–4 hours after pollination the first pollentubes are found in the style.

The profiles for the incorporation of ^{14}C-leucine and for the amount of ribosomal proteins from ovaries of clone T2U differ in two respects from those of W166K (DEURENBERG 1976). First, there is a difference at the very beginning. In

T2U, all profiles start with an increase, whereas in W166K there is a decrease. When one starts the experiments 3-4 hours later and at the same time assumes that before the moment of emasculating there was an increase and subsequently a decrease in incorporation activity and content of ribosomal proteins, in all experiments the profiles would start with a decrease.

The difference at the end of the described experiments, e.g. the decline of the profiles of W166K in contrast with the experiments with T2U, is more difficult to explain. One can speculate about a process of "conditioning" of the tissues of the ovaries necessary for a good reception of the male gametes. In the case of W166K the ovary has to wait after this conditioning for the pollentubes which arrive a little later because of the longer style in W166K. However, in T2U the processes of conditioning and fertilization are better coordinated with regard to timing. Perhaps this process of conditioning could be a process for overcoming a second barrier for the pollentubes. This second barrier for self-pollentubes supposedly only can be broken down after a signal arriving from the stigma or the style after cross-pollination.

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The author is much indebted to Prof. Dr. H. F. Linskens for his stimulating interest, to Dr. Ir. W. G. M. Barendse for reading and correcting the manuscript and to Mrs. E. R. Tummers-Moesker for typing the manuscript.

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Planta

Differentiated Protein Synthesis with Polysomes from *Petunia* Ovaries before Fertilization

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Abstract. Polysomes from cross-, self- and unpollinated ovaries have been injected in *Xenopus laevis* eggcells. The synthesised proteins have been identified by means of gel-electrophoresis. It appeared that few hours after pollination the protein pattern of the pollinated ovary has changed in comparison with that of unpollinated ones. However, in this early stage, there is only little difference found in the protein pattern of selfed and crossed ovaries. From 12 h after pollination and onwards, the difference between pollinated and unpollinated ovaries becomes very obvious, as does also the difference between crossed and selfed ovaries. At 24 h after pollination many different proteins exist in crossed and selfed ovaries. From 32 h to 48 h after pollination the crossed ovaries differentiate further on, whereas the ovaries after self-pollination show more and more proteins which were also found in the unpollinated ovaries of that age. Possible explanations and hypothesis for these phenomena are discussed as well as the probable occurrence of signal(s) arriving from the stigma or the style at the ovary.

Key words: Fertilization – Ovaries – *Petunia hybrida* – Polysomes – Protein synthesis – *Xenopus laevis*

Introduction

The protein metabolism of the ovary shows differences after cross- and self-pollination. Polysomes from ovaries after cross- and self-pollination give rise to different stimulating activities in the in vitro incorporation of ^{14}C-leucine, as compared with polysomes from unpollinated ovaries (Deurenberg, 1976a and 1976b). This suggests, that in crossed and selfed ovaries possibly several m-RNA's are translated. To investigate this possibility, the proteins coded for on the m-RNA, must be isolated and identified. Several authors have worked successfully with the *Xenopus laevis* in vivo translation system for the study of translation of m-RNA from zoological origin (Gurdon et al., 1971; Marbaix et al., 1972; Moar et al., 1971, Lane et al., 1971). The translation of plant m-RNA in eggcells of *Xenopus* (African clawed toad) with subsequent biological testing of the synthesised proteins has been reported by van der Donk (1975a and 1975b), using the same system.

We have injected polysomes from *Petunia* ovaries together with labelled amino-acids in unfertilized eggcells of *Xenopus laevis*. After incubation, the newly synthesised proteins were isolated and identified with SDS-ureum gel-electrophoresis.

Materials and Methods

The flowers of *Petunia hybrida* plants, clone W166K, incompatibility alleles S_1S_2, grown in the greenhouse were emasculated and pollinated with their own pollen (self-pollination) and with pollen from clone T_2U (incompatibility alleles S_3S_4) cross-pollination. Non-pollinated flowers served as a control. The ovaries were collected and the polysomes extracted as described previously (Deurenberg, 1976a).

Unfertilized *Xenopus laevis* eggcells were obtained the day after injecting a female with 600 I.U. of pregnyl. The eggs were treated as mentioned by van der Donk (1975a).

Polysomal material (corresponding to ±20 ng RNA) was injected with 50 nCi [^{3}H]leucine (spec. act. 250 mCi/mmol) in a total volume of 30 nl injection medium (Gurdon et al., 1971). Controls were injected in the same way except for the addition of polysomes. The injection was carried out with a device for microinjection described by de Laat et al. (1974). After incubation for about 18 h at 17° C (Gurdon et al., 1968; van der Donk, 1975a) the injected eggcells were homogenised in 0.5 ml Tris/HCl buffer 50 mM, pH = 7.6, containing 5 mM Mg acetate, 25 mM NH_4Cl, 6 mM β-mercapto-ethanol and 2% SDS (sodium dodecyl sulphate). The homogenate was finally taken up in 2 ml of this solution. The proteins were precipitated with an equal volume of 20% ice-cold TCA (trichlor acetic acid) and centrifuged down at 1500 × g

for 5 min The pellet was subsequently washed in 10% TCA, heated at 90° C for 5 min, cooled and again centrifuged The pellet was dissolved in 0 1 ml per egg of 0 01 M phosphate buffer pH=7 0 containing 0 1% SDS and 0 1% β-mercapto-ethanol. The proteins were complexed with SDS during 2 h at 37° C

SDS-ureum Gel-electrophoresis

Disc-electrophoresis was carried out in 10% polyacryl-amid gels at 2 mA per gel in the presence of 4 M urcum, 0 1% SDS in 0 05 M Tris/HCl pH=8 3 In all runs "cold" cytochrome c (MW= 12,500) was added as a marker The gels were cut into slices of 1 mm (Micle Gel Slicer) and counted in a Philips Scintillation Counter in the following scintillation fluid 5 5 g Scintimix III, 30 ml Soluene-350, 20 ml 1,2-dimethoxy-methane per liter toluene

Sucrose-density Gradient

Sucrose-density gradients (20 40%) were carefully layered with 0 1 ml of the incubation medium containing the polysomes After centrifugation, the tubes were punctured and the gradient was measured in a continuous flow cuvet (Deurenberg, 1976a)

Chemicals

[^{3}H]leucine, spec act 250 mCi/mmol, was purchased from The Radiochemical Centre, Amersham Strepto-mycine sulphate and penicilline-G were obtained from Sigma Chemicals Actinomycin-C_1 and cytochrome c came from Boehringer and Sohne, GmbH, Mannheim Scintimix III is a product from Merck and Soluene-350 from Packard

Results

After gel-electrophoresis the gel was sliced into 1 mm discs and the radio-activity per gel-slice in desintegrations per minute was counted as percentage of the total activity per gel. The mobility was expressed as percentage of the migration of cytochrome c.

The Figures 1–6 show the relative incorporation activities of the slices as a function of the relative migration distances. Curve B in Figure 1 gives the proteins which were labelled after injection of [^{3}H]leucine with injection medium in *Xenopus* eggcells. This curve served as a reference. The proteins synthesised migrated in the gels to 0.4–0.7 and 1.0–1.2. The total incorporation activity of this control injection was however, in a maximum about 10% of the total incorporation after injection of injection medium with polysomes.

The synthesis pattern after injection of polysomes from ovaries at zero time is given in curve U of Figure 1. This represents the basic activity of the ovary.

After pollination the incorporation activity of the ovaries changed dramatically. The Figures 2–6 show a shift in the overall molecular weight of the newly synthesised proteins to the lower region of the scale,

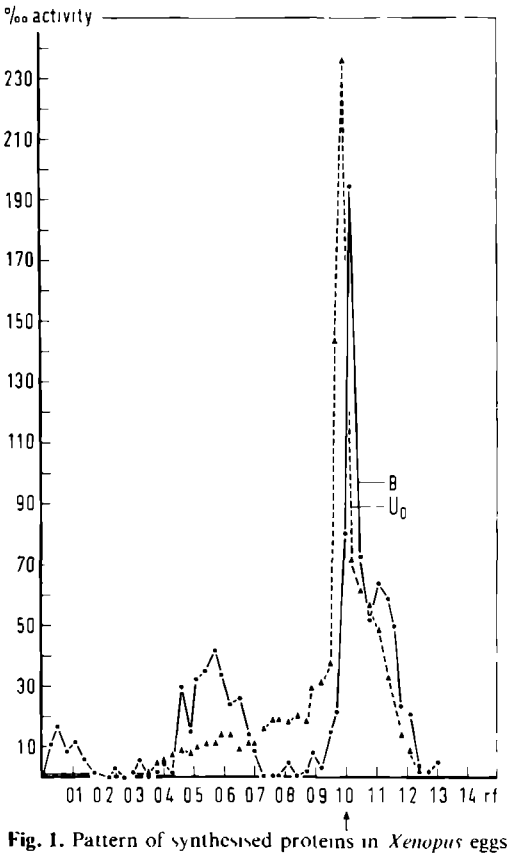

Fig. 1. Pattern of synthesised proteins in *Xenopus* eggs after reference – injection (*B*) and injection of polysomes from ovaries at zero time (*U*) See also legenda Figure 2 and "Material and Methods"

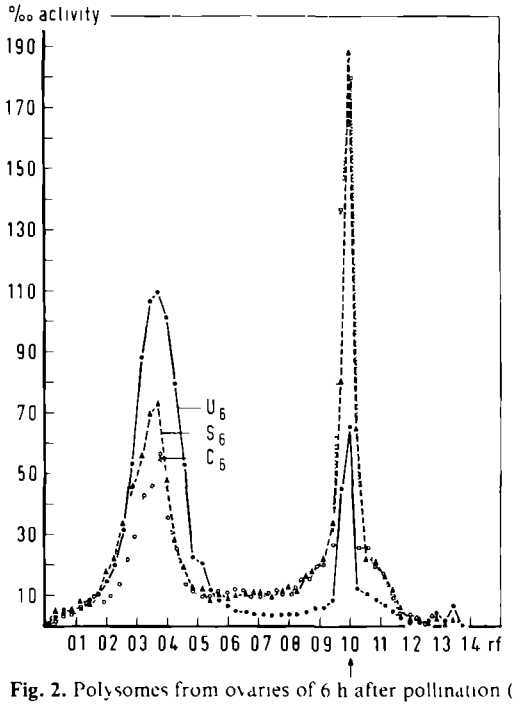

Fig. 2. Polysomes from ovaries of 6 h after pollination (self-, cross- and nonpollination) were injected in *Xenopus* eggs The synthesised proteins were separated with SDS-ureum gel-electrophoresis The gels were sliced and the radio-activity measured See Material and Methods

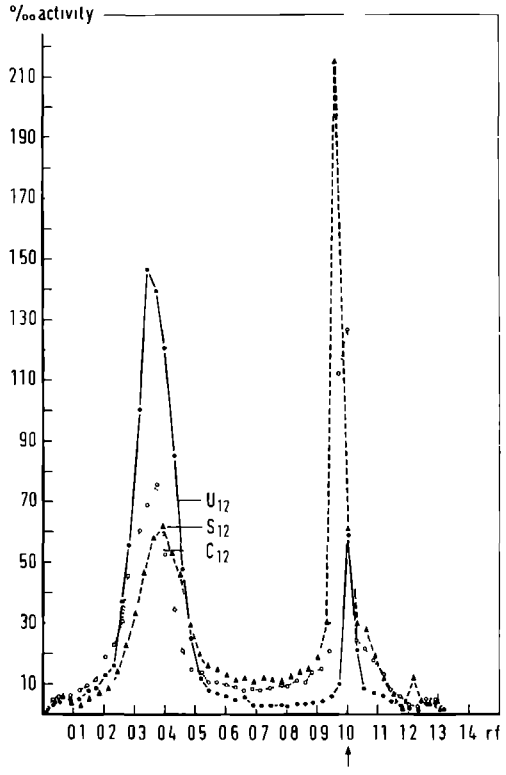

Fig. 3. As Figure 2 except for 12 h after pollination

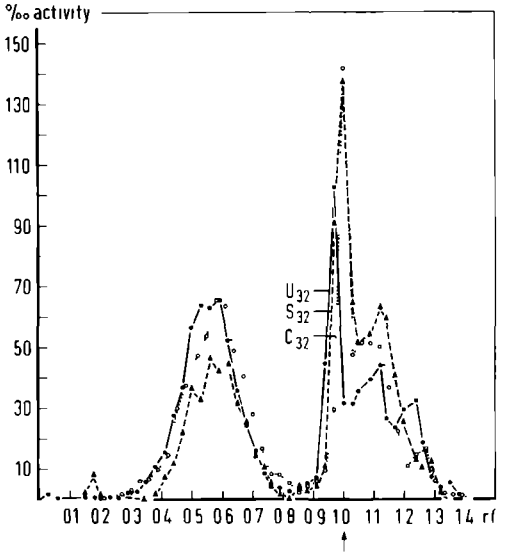

Fig. 5. As Figure 2 except for 32 h after pollination

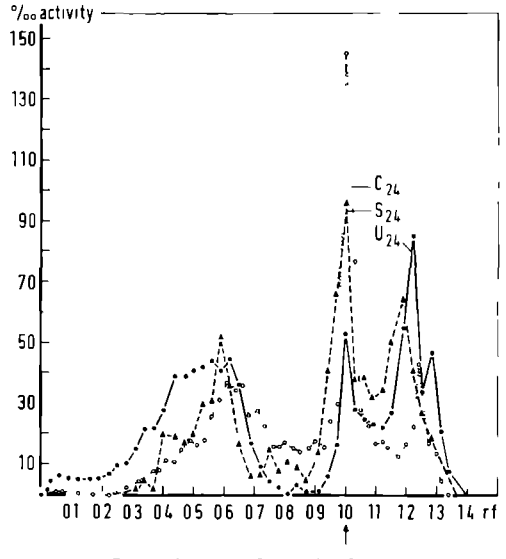

Fig. 4. As Figure 2 except for 24 h after pollination

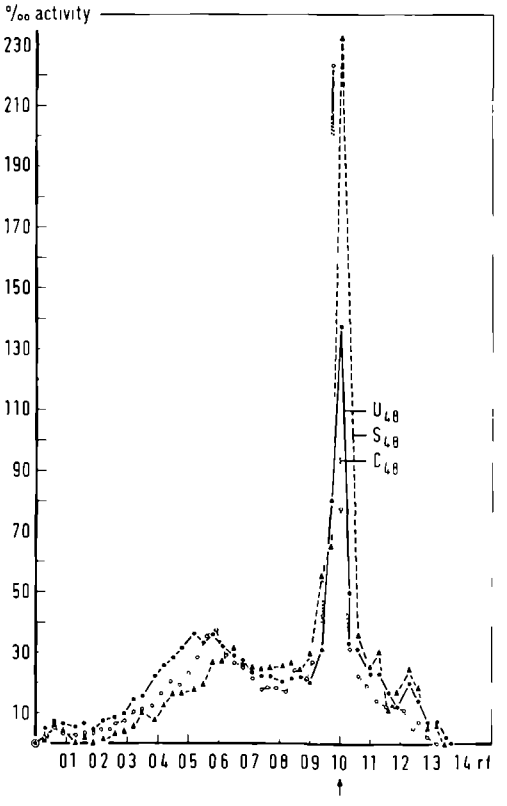

Fig. 6. As Figure 2 except for 48 h after pollination

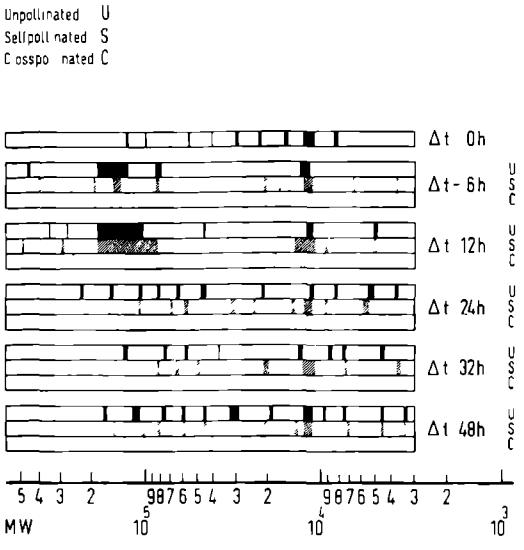

Fig. 7 Protein synthesised in *Xenopus* eggs after injection of polysomes from self cross and unpollinated ovaries at different times after pollination

expressed as a shift to higher Rf values We can distinguish roughly 2 groups of proteins one group with mol weights of about 40,000 200 000 (between Rf= 0 3–0 7) and a second group with a mol weight of about 12,000 dalton (Rf=1 0)

At 6 and 12 h after pollination a difference was observed in the protein composition after pollination and nonpollination (Figs 2 and 3) This difference consisted not only of a changed ratio between the total activities of the high and low mol weights proteins, but also of differences in the individual proteins within both groups This is better demonstrated in Figure 7, where the newly synthesised proteins are given with their approximate mol weights Comparing the Figures 2 and 7, e g 6 h after pollination, we see a slight difference between cross- and self-pollinated ovaries Many proteins of the cross-pollinated ovaries show the same relative mobility in the gels as proteins from self-pollinated ovaries There were only a few proteins which were different in both cases e g 9,000, 50,000, 300,000 and 500,000 dalton

So, whereas the difference between pollinated and unpollinated ovaries has been established, only small differences could be found between cross- and self-pollinated ones

At 12 h after pollination the difference between the unpollinated on the one hand, and the pollinated ovaries on the other hand was even more obvious than after 6 h after pollination (Fig 3) The differences after cross- and self-pollination had become more pronounced The relative incorporation of [^{3}H]leucine in proteins of mol weight of about 12,000 dalton was very much higher after self-pollination than after cross-pollination In both cases the incorporation activities at 12,000 dalton were very much higher than those after non-pollination Figure 7 demonstrates that at 12 h after pollination only a few proteins showed the same relative mobility after self- and after cross-pollination, namely 2 or 3 proteins having mol weights of about 12,000 and 500,000 daltons These 3 proteins of about 12,000 dalton were also found at 24 h after cross- as well as after self-pollination

In the region of Rf=0 3–0 5 (90,000 200,000 dalton) the differences between the unpollinated and pollinated ovaries were very obvious Whereas there was a relatively high incorporation in the unpollinated ovaries, the relative activities after self- and cross-pollination were moderate However, more differentiation in the individual proteins was observed (Fig 7)

The region between 12,000 and 90,000 dalton (Rf=0 5 0 9) is difficult to interpretate since only very weak differences existed after pollination More clear seemed the situation with regard to proteins of a mol weight of about 100,000–500,000 dalton (Rf=0 5 and onwards) After self-pollination we see a very broad peak at 100,000 with little differentiation (Figs 3 and 7), and 2 peaks at 300,000 and 500,000 dalton After cross-pollination, there were several peaks or shoulders found in this region and thus more differentiation in the individual proteins At 24 h after pollination the difference between unpollinated self- and cross-pollinated ovaries was very clear (Fig 4) The pattern was very complex The differences between unpollinated and pollinated ovaries were obvious After nonpollination the region of high mol weights proteins showed a wider distribution of the single proteins than after pollination After nonpollination there was a minor peak at 12,000 (Rf=1 0) and a very pronounced peak at Rf=1 2 as compared with cross- and self-pollination, which had a more pronounced and minor peak at these Rf values respectively Looking at differences between crossing and selfing, we see that in the region of 30,000–200,000 dalton there were much individual proteins after cross-pollination, which were not found after self-pollination After selfing the more pronounced proteins were found between 60,000 150,000 dalton, whereas after crossing this region was more dense 35,000–150,000 dalton The low mol weight proteins at 3,000–12,000 dalton showed more incorporation after selfing than after cross-pollination The highest incorporation activities were found at different places after selfing at 55 000 and after crossing at 45,000 dalton

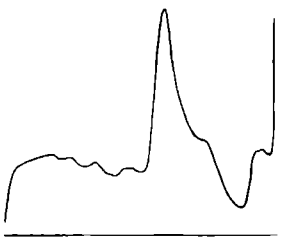

Fig 8 Distribution of polysomes on sucrose density gradient

At 32 h after pollination the differences between the different treated ovaries changed further In the unpollinated ovaries the relative importance of the peak at Rf=1 2 diminished and again the proteins at Rf=1 0 showed the highest incorporation The incorporation at Rf=0 4–0 6 (60,000 100,000 dalton) increased In the self-pollinated ovaries the peak at Rf=1 2 (5,000 dalton) disappeared and a new one was found at Rf=1 1 (8,000 dalton) which have also been found in the unpollinated ovaries Also changes were found in the region Rf=0 4–0 6 in relation to the situation at 24 h after pollination

The cross-pollinated ovaries changed further in all regions At 48 h after pollination (Fig 6) the low mol weight proteins showed still clear differences between unpollinated and pollinated ovaries The high mol weight peaks were of the same magnitude in all three cases, but showed still slightly different incorporation bands Comparing the incorporation bands of unpollinated and self-pollinated ovaries (Fig 7), we see that several proteins occurred in both types of ovaries the fractions of 3500, 4500, 12,000, 45,000, 58 000 dalton The incorporation activity, however, had not the same level in both types of ovaries, the unpollinated ovaries contained generally less proteins

The cross-pollinated ovaries showed only a few bands which occurred also in the selfed and in the unpollinated ovaries So, whereas there was a similarity between selfed and unpollinated ovaries, the cross-pollinated ones showed a pattern that differed in many respects from the unpollinated ovaries

In order to make sure that the material injected in *Xenopus* eggs were polysomes, the injection medium containing the pellet after ultra-centrifugation, was layered on a sucrose gradient and centrifuged After measurement of the distribution on the gradient the polysomal pattern was found as is demonstrated in Figure 8 We see that little if any degradation of the polysomal material has occurred in the injection medium So, it seemed to be justified to conclude that indeed polysomes had been injected into the egg-cells and not any striped form of polysomes

Discussion

A difference in the protein pattern is observed between unpollinated and pollinated ovaries as early as at least 6 h after pollination A difference between self- and cross-pollinated ovaries can be determined after 12 h These results indicate, that previous conclusions (Deurenberg, 1976a) are now firmly established Long before pollentube tips reach the base of the style, the ovary seems to change the metabolism to differentiated protein synthesis crossed and selfed ovaries synthesise different proteins, also compared to the unpollinated ovaries As has already been concluded previously (Deurenberg, 1976a), the difference between pollinated and unpollinated ovaries can be established 6 h after pollination the total amount of proteins synthesised increases which can also be interpretated as an altered differentiation in the population of m-RNA's, compared to the unpollinated ovaries This altered population of mRNA's results in a changed pattern of proteins after gel-electrophoresis In this paper we could discriminate between cross- and self-pollination about 12 h after pollination The difference occurring already weakly at 6 h, leads, however, to a clear difference in the pattern of proteins on the gels at 12 h, later on, also a change occurs in the amount of synthesised proteins (Deurenberg, 1976a)

The later after pollination, the more obvious the differences between cross- and unpollinated ovaries become This can be understood as a conditioning of (some) tissues of the ovary in order to give an optimal development of the embryo It also may explain the resemblance of the protein pattern of the selfed and unpollinated ovaries at 48 h after pollination Because the selfed ovaries did not receive pollentubes and thus no embryo can be formed, the tissues slowly degenerate as do the tissues of the unpollinated ones At 48 h after self-pollination many proteins were of the same size as after nonpollination Perhaps these proteins are of enzymatic nature and have a function in the break-down of the tissues

From the Figures it becomes clear that there must be a long distance transmission of information from the style or the stigma to the ovary One could speculate about the existence of two subsequent signals The first signal gives information to the ovary about the fact whether or not pollination has taken place As a consequence, the ovaries alter their metabolism as compared to unpollinated ones (Figs 2 and 7)

At this stage there exist not yet differences between the cross- and the self-pollinated conditions At a later stage estimated at about 4 h after pollination, a second signal arrives, which gives additional information about the kind of pollination, own of foreign

The crossed ovaries differentiate further to develop to the optimal conditions for receiving the male gametes. The selfed ovaries develop slower or "back" to the metabolic state which is characteristic for the unpollinated ovaries of this age.

About the nature of the long distance signal(s) we only can speculate at this moment. Electrical signals are in discussion. Fast diffusion of signal-substances, diffused from the pollengrain and (or) the initial pollentube are possible but have not yet been demonstrated.

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EVIDENCE AGAINST THE FORMATION OF FAST DIFFUSING SUBSTANCES PRECEDING FERTILIZATION IN PETUNIA.

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Differentiated protein and RNA metabolism in the ovaries of *Petunia* after cross- and self-pollination has been described previously (LINSKENS 1973, DEURENBERG 1976a, b, 1977). It was observed that the differentiation was altered after pollination long before the tips of the pollentubes reached the ovary. From this observation was concluded that recognition signals had to be emitted from the stigma or the style in order to evoke the differential metabolic processes which result in a differentiated protein metabolism with regard to the total amounts of proteins synthesised as well as the protein pattern.

Two possible explanations were supposed for these phenomena: and electrical signal (LINSKENS & SPANJERS 1973) and fast diffusing substances. Fast diffusing substances are known in the plant kingdom in relation to rhemonastic movements as well as phototropic reactions (UMRATH 1959, LEA 1976).

The style of *Petunia* has two small vascular bundles, differentiated in xylem and phloem which may serve as possible pathways for the transport of the hypothetic signal substances (KONAR & LINSKENS 1966, CANNY 1975; FENSOM 1975). Another possible way of transport are the conductive tissues.

This paper deals with experiments which show evidence against the existence of long distance, fast diffusing signal substances. Pollengrains, labelled with ^{3}H-leucine were brought on the stigma of the *Petunia* flowers. The distribution of the label over the style and the ovary was measured at different time intervals. The *figs. 1* and *2* show that some radio-activity was brought into the stigma, together with the pollen. It can be supposed that the labelled leucine is incorporated into the pollen grains as well as absorbed to the pollen grain-wall (LINSKENS 1959).

In *fig. 1* the distribution of the label over the style, stigma and ovary is shown at 30 minutes, 2 and 4 hours after pollination. In each case the counts per minute had been determined from 2 equivalent pieces of the style or of two stigmas or ovaries.

The distribution of the label over the style is in the same order as the background activity. From this we conclude that no labelled substances were diffused out of the pollen into the style.

A control experiment has been carried out in which a droplet of ^{3}H-leucine solution was brought on the surface of the stigma. Again at several time intervals after administration of the label the style was cut into 2 mm pieces and counted. No diffusion of label over the style was observed. The number of

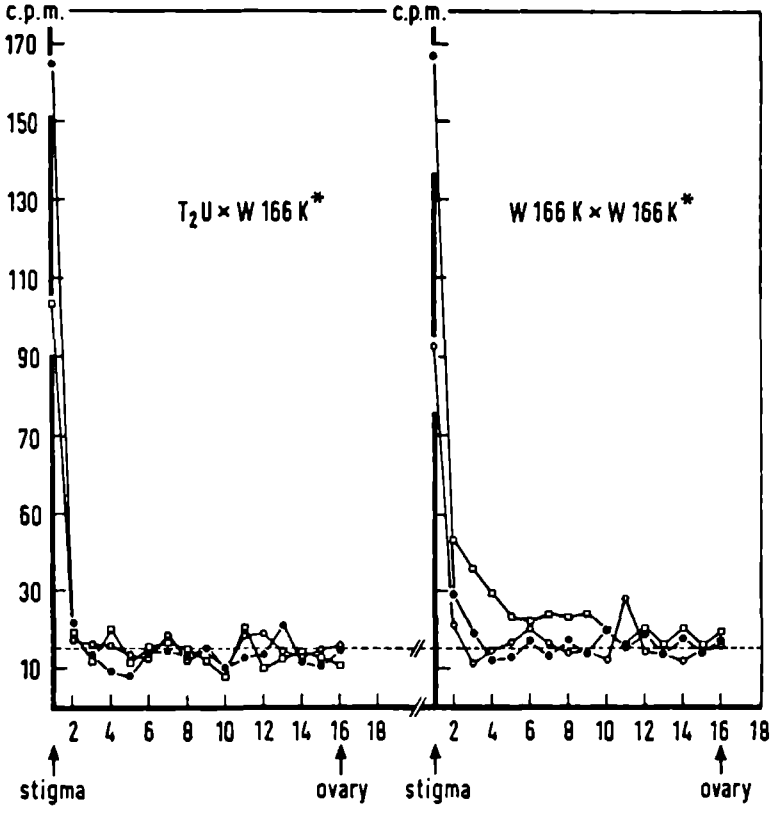

Fig. 1. *Petunia* buds, length of ± 30 mm, from which the corolla and the anthers had been removed, were placed in a vial with a solution containing 1 µl of ^{3}H-leucine (spec. act. 250 mCi/mmol) in 1 ml of water. After incubation for 48 hours at 18°C the pollen was collected, dried and stored in a refrigerator at -20°C.

From buds of 24 hours before anthesis, clone W166K (incompatibility alleles S_1S_2, fig. 1) and clone T_2U (S_3S_3, fig. 2), the petales and the anthers were removed and the remaining ovary, style and stigma with the receptacle was pollinated with the labeled pollen and placed in a vial with a little water. After different time intervals the stigma and the ovary were removed and the style was cut into pieces of 2 mm. All pieces were put into scintillation vials containing 1 ml of Soluene-100 and incubated during 2 hours at 56°C for digestion of the tissues. Ten ml of toluene, PPO and POPOP solution was added and the radio-activity was measured in a Philips Liquid Scintillation Analyser.

□ 30 minutes after pollination.
○ 2 hours after pollination.
● 4 hours after pollination.

counts in each piece of the style did not significantly exceed the background activity.

These results indicate that no substances labelled with ^{3}H-leucine nor ^{3}H-leucine itself possessed the property of fast diffusion and thus can not function

in forwarding information about the kind of pollination from the style or the stigma to the ovary.

These results are in agreement with those of experiments in which labelled proteins were injected in various ways into the style, and/or in which labelled proteins were administered to the surface of the stigma. In those experiments a diffusion of 1 cm per 24 hours had been found (van der Donk, pers. comm.). Such a diffusion is not fast enough since the changes in the protein metabolism of the ovary already take place within a few hours after pollination (DEURENBERG 1976 a,b, 1977).

Although the above mentioned experiments do not exclude the existence of fast diffusing signal substances, it seems that the alternative possibility of signals of an electrical nature is more probable. There are indications that such phenomena can be shown in the style of *Petunia* (LINSKENS & SPANJERS 1973, SINYUKHIN & BRITIKOV 1967).

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Inkompatibiliteit, het onvermogen van een fertiele hermafrodiete zaadplant om na zelfbestuiving zygoten te vormen (de Nettancourt 1977), is tot nu toe bij Petunia slechts gezien als een fenomeen dat zich alleen in de stijl zou afspelen.
Hoewel aangetoond werd dat het vruchtbeginsel en andere organen van de bloem in een zeer vroeg stadium kunnen reageren op de bestuivingsprikkel door het op gang brengen van aktiveringsprocessen, is het mogelijke verband tussen inkompatibiliteit en de reactie van het totale vruchtbeginsel op de bestuiving nog niet onderzocht.

Nitsch (1965) maakte in de regulatie van de groei van vruchten onderscheid tussen het effect van de bestuiving en dat van de bevruchting. Zoals uit vele oude literatuurgegevens blijkt, zijn als resultaat van alleen bestuivingen zonder daarop volgende bevruchtingen, bij veel planten vruchten zonder zaad te verwachten. Gustafson (1942) suggereerde dat voor de aktivering van het vruchtbeginsel tot de vorming van de vrucht zonder voorafgaande bevruchting, groeistoffen nodig zijn. Deze zouden in eerste instantie gevormd worden na een bestuiving, ook als het een bestuiving betrof met pollen, waarvan geen bevruchting te verwachten is, zoals bij de vorming van vele zaadloze vruchten.
Bij planten, die zaadloze vruchten kunnen leveren, zou deze hoeveelheid groeistof voldoende zijn voor het stimuleren van het vruchtbeginsel tot de vorming van de vrucht. Bij de andere planten zou als gevolg van de bevruchting, volgende op de effecten van de bestuiving de produktie van groeistoffen in stand worden gehouden, zodat na een eerste aanzet daartoe, de vorming van de vrucht verder kan verlopen.

Dit onderzoek heeft getracht aanwijzigingen naar voren te brengen over een verband tussen het inkompatibele of kompatibele karakter van de bestuiving en de biochemische veranderingen in het eiwit-metabolisme van het vruchtbeginsel na de bestuiving, echter vóór het tot stand komen van de dubbele bevruchting.

De hoeveelheid polysomen, welke uit vruchtbeginsels geïsoleerd kon worden, bleek reeds ± 9 uur na de bestuiving veranderd te zijn in vergelijking met vruchtbeginsels van onbestoven bloemen. Ook het vermogen van deze polysomen om in een in vitro systeem eiwitten (polypeptiden) te synthetiseren, vertoonde na bestuiving een sterke stijging vergeleken met polysomen uit vruchtbeginsels van onbestoven bloemen. Deze verschillen bleven aantoonbaar tot 66 uur na bestuiving.
Tussen 12 en 24 uur na bestuiving konden ook veranderingen tussen de vruchtbeginsels van kruis- en zelfbestoven bloemen worden aangetoond. in het vermogen tot in vitro incorporatie van aminozuren door de geïsoleerde polysomen. Aangezien de eerste pollenbuizen pas omstreeks 50 uur na kruisbestuiving het vruchtbeginsel bereiken, na een zelfbestuiving nog niet na 70 uur, moet er één of andere vorm van informatie-overdracht aan de pollenbuizen vooraf gegaan zijn. Deze informatieprikkel(s) zorgt (zorgen) niet alleen voor de verandering in samenstelling en hoeveelheid polysomen tussen vruchtbeginsels van bestoven en onbestoven bloemen, maar zal (zullen) ook het verschil in reaktiviteit na zelf- en kruisbestuiving moeten kunnen bewerkstelligen (Deurenberg 1976a).

Het effect van bestuiving op de polysomen populatie kon ook gedemonstreerd worden bij Petunia planten met een geheel andere genetische samenstelling, een andere kloon, nl. T_2U met de S-allelen S_3S_3 in plaats van kloon W166K met de inkompatibiliteits-allelen S_1S_2.
Ook hier was er na ± 9-12 uur na bestuiving een duidelijk verschil te zien vergeleken met de vruchtbeginsels van onbestoven bloemen, zowel een vermeerdering van het polysomale materiaal als ook een verhoogde incorporatie activiteit van aminozuren in polypeptiden.
Een verschil in reaktiviteit na zelf- en kruisbestuiving was ook aantoonbaar: tussen 18 en 30 uur na bestuiving vertoonden vruchtbeginsels na kruisbestuiving een hoger gehalte aan polysomaal materiaal als ook een hogere incorporatie activiteit dan na zelfbestuiving. Ook hier konden de resultaten slechts tot de konklusie leiden, dat er informatie vanuit stigma of stijl naar het vruchtbeginsel doorgegeven werd, waarna het eiwit-metabolisme van de vruchtbeginsels veranderde.
De genetische achtergrond (met uitzondering van de S-allelen) heeft hierop dus blijkbaar geen invloed. Waarschijnlijk is er slechts van belang, dat er een " prikkeling " door een bestuiving is geweest en

het kompatibele of inkompatibele karakter van de bestuiving (Deurenberg 1976b).

Ook kwalitatief gezien loopt het eiwit-metabolisme van de vruchtbeginsels al dan niet na bestuiving, na kruis- of zelfbestuiving, zeer sterk uiteen. Reeds zeer vroeg, 6 uur na bestuiving, is het eiwit-metabolisme, wellicht ook het RNA-metabolisme, veranderd. In dit vroege stadium zijn de verschillen tussen kruis- en zelfbestuiving nog niet erg groot: slechts enkele differentiële eiwitten treden op. Vanaf 12 uur na bestuiving worden de verschillen steeds duidelijker. Terwijl de vruchtbeginsels zich na een kruisbestuiving verder differentiëren, zien we dat 32 en 48 uur na een zelfbestuiving het eiwit-patroon steeds meer gemeenschappelijke proteïnes laat zien met de vruchtbeginsels van onbestoven bloemen. Hieruit zou gekonkludeerd kunnen worden dat de vruchtbeginsels na zelfbestuiving eerst dezelfde aktiveringsprocessen doormaken als de vruchtbeginsels na kruisbestuiving, maar daarna dezelfde processen vertonen als de vruchtbeginsels van onbestoven bloemen (Deurenberg 1977a).

In een poging om enige aanwijzing te verkrijgen over de identiteit van de informatiestroom vanuit het stigma of de stijl, na kruis- en zelfbestuiving, zijn er experimenten gedaan met radio-actief leucine gemarkeerde pollen (Deurenberg 1977b). Hierbij werd gehoopt, dat er gemarkeerde stoffen uit de pollenbuizen of pollenkorrels zouden diffunderen en via de stijl in zeer korte tijd naar het vruchtbeginsel getransporteerd zouden worden. Dit zou het chemisch karakter van deze informatiestroom aangetoond hebben. De resultaten waren negatief. Dit betekent, dat het informatie overbrengend agens, óf niet gemarkeerd kan worden met leucine (misschien wel onder andere omstandigheden), óf dat het agens van geheel andere aard is, bijvoorbeeld een elektrisch signaal.

De processen, welke in de vruchtbeginsels van Petunia tot $\pm$ 12 uur na kruis- en zelfbestuiving verlopen, kunnen beschouwd worden als dezelfde aktiveringsprocessen van het vruchtbeginsel. Voor het op gang brengen van deze processen kan zowel kompatibel als inkompatibel pollen gebruikt worden. Hieruit, en uit het feit dat tot 12 uur na bestuiving nog geen sprake van een bevruchting kan zijn, mag wellicht afgeleid worden, dat het hier aktiveringsprocessen betreft, welke

alleen het gevolg zijn van een bestuivingseffect in de zin van Nitsch (1965). Misschien kunnen deze processen dan ook gezien worden als een eerste aanzet tot de vorming van de vrucht. Deze processen stoppen dan blijkbaar na verloop van tijd na een zelfbestuiving, terwijl na een kruisbestuiving er een verdere ontwikkeling plaats zal hebben. Deze verdere ontwikkeling is dan afhankelijk van de herkenningsreaktie in de stijl dat het een kompatibele bestuiving betrof, en later van de uiteindelijke bevruchting. Of er bij Petunia een zelfde regelmechanisme met groeistoffen voor de vruchtvorming bestaat als voor de vorming van vruchten bij andere plantensoorten volgens het model van Gustafson, kan betwijfeld worden gezien de resultaten van onderzoek naar groeistoffen o.a. in vruchtbeginsels van Petunia (Barendse et al. 1970). Zij konden geen significante verschillen in hoeveelheden groeistoffen in vruchtbeginsels al dan niet na bestuiving aantonen.

De in dit proefschrift geschetste aktiveringsprocessen zouden ook een funktie kunnen hebben bij de drastische veranderingen in het transport en de synthese van stoffen, welke in de bloem na een bevruchting zullen gaan optreden. Tal van stoffen zullen dan gebruikt worden voor de opbouw van de vrucht en het zaad. Alle bruikbare materialen uit de verwelkende bloembladeren, antheren en stijl, zullen naar het vruchtbeginsel getransporteerd worden. Wellicht ook spelen de eiwitten, gevormd bij de aktiveringsprocessen, een rol bij de synthese van veel belangrijke bestanddelen van het zich ontwikkelende zaad en de vrucht.

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Samenvatting

1. Na kruisbestuiving van bloemen van Petunia hybrida, kloon W166K, werden uit de vruchtbeginsels de polysomen geëxtraheerd. De polysomen na kruis- en zelfbestuiving vertoonden in een in vitro systeem voor eiwit-synthese een grotere incorporatie activiteit voor aminozuren dan de polysomen uit vruchtbeginsels van onbestoven bloemen. Dit verschil was reeds aantoonbaar voor de pollenbuizen de vruchtbeginsels hadden bereikt. Ook verschilde de incorporatie activiteit door polysomen na kruisbestuiving van die van polysomen na zelfbestuiving.

2. Polysomen uit vruchtbeginsels van Petunia hybrida, kloon T_2U, vertoonden na kruis- en zelfbestuiving een verhoogde incorporatie activiteit van radioactief gemarkeerde aminozuren vergeleken met die van polysomen uit onbestoven bloemen. Dit effect werd gevonden op een tijdstip waarop de pollenbuizen het vruchtbeginsel nog niet bereikt konden hebben. Ook hier kon gekonkludeerd worden, dat er een verschil in eiwit-synthese na kruis- en zelfbestuiving optrad, nog voor de pollenbuizen in het vruchtbeginsel waren aangekomen.

Uit de bovenstaande resultaten (1 en 2) werd gekonkludeerd, dat er bij Petunia, na bestuiving informatie overdracht vanuit het stigma of de stijl naar het vruchtbeginsel plaats vindt. Dit signaal bereikt het vruchtbeginsel al eerder dan de pollenbuizen en bevat blijkbaar informatie over het feit van de bestuiving, alsmede over de aard, kompatibel of inkompatibel van de bestuiving.

3. Polysomen uit vruchtbeginsels na kruis- en zelfbestuiving, als ook uit vruchtbeginsels van onbestoven bloemen werden in onbevruchte eieren van Xenopus geïnjecteerd. De in deze eieren gesynthetiseerde eiwitten konden geëxtraheerd en met behulp van gel-elektroforese gescheiden worden. Reeds enkele uren na kruis- en zelfbestuiving was het eiwit-patroon anders dan het patroon bij onbestoven bloemen. In dit stadium verschilden echter de patronen na kruis- en zelfbestuiving nog niet significant van elkaar. Vanaf 12 uur na bestuiving werden de verschillen tussen vruchtbeginsels van bestoven en onbestoven bloemen zeer duidelijk en kon ook een onderscheid gemaakt worden tussen de banden-patronen na kruis- en na zelfbestuiving. Later, 24 uur na bestuiving, werd een grote verscheidenheid in eiwitten na kruis- en zelfbestuiving in de gels gevonden.

Terwijl het eiwit-patroon na kruisbestuiving zich verder differentiëerde vanaf 32 uur na bestuiving, vertoonde het patroon na zelfbestuiving vanaf dit tijdstip steeds meer gelijkenis met dat van onbestoven bloemen.

4. Na bestuiving met radio-actief gemarkeerde pollen kon geen " stroom " van radio-actieve stoffen vanuit het pollen door de stijl aangetoond worden. Deze bevinding kan beschouwd worden als een ondersteuning van de hypothese dat het hierboven bedoelde signaal geen stof is waarin leucine ingebouwd is.

Curriculum vitae.

Jan Jozef Maria Deurenberg werd op 20 januari 1946 in Kerkrade geboren. Daar doorliep hij de Lagere School en behaalde in 1963 het H.B.S.-b diploma aan de St. Anthonius Dr. H.B.S. In dat zelfde jaar begon hij aan de Katholieke Universiteit in Nijmegen met de biologie-studie. Het kandidaatsexamen werd in oktober 1966 afgelegd. De na-kandidaatsstudie bestond uit het hoofdvak Chemisch Cytologie o.l.v. Prof. Dr. Ch. M. A. Kuyper. Tijdens het bewerken van dit hoofdvak werd stage gelopen aan het Zoologisch Instituut van de Universiteit van Bern (Zwitserland) o.l.v. Prof. Dr. R. Weber. De bijvakken waren Botanie en Zoologie, welke respectievelijk uitgevoerd werden o.l.v. Dr. Ir. J.F.G.M. Wintermans en Dr. F.S. Lukoschus. Het doktoraalexamen werd in september 1969 afgelegd, waarbij tevens onderwijsbevoegdheid werd verkregen. Na de militaire dienstplicht had hij vanaf augustus 1971 tot januari 1976 een tijdelijke aanstelling als wetenschappelijk medewerker aan het Botanisch Laboratorium te Nijmegen. Inmiddels was hij in september 1971 in het huwelijk getreden met H.W.J. Vos. In mei 1976 werd aan het Instituut voor Toepassing van Atoomenergie in de Landbouw (ITAL) met goed gevolg het examen als bewijs van bekwaamheid voor het beheren van een C-lab afgelegd.
Vanaf augustus 1976 tot augustus 1977 was hij docent aan het Instituut voor Hoger Beroepsonderwijs te Eindhoven.

STELLINGEN

1. Bij het inkompatibiliteitsonderzoek van Petunia is tot nu toe het vruchtbeginsel ten onrechte buiten beschouwing gebleven. (Dit proefschrift)

2. Aangezien de geslachtscel-differentiatie bij bedektzadigen achterwege blijft, dient de rol van deze geslachtscellen tijdens de bestuiving en bevruchting nauwkeurig omschreven te worden.
M.T.M. Willemse: Vernieuwing en Verscheidenheid, L.H.Wageningen (1976)

3. Bij het beheren van landschappen en natuurreservaten dient meer gehandeld te worden in de geest van de relatietheorie, opgesteld door van Leeuwen.
Ch.G. van Leeuwen: Gorteria 2(8), 93 (1965)
Ch.G. van Leeuwen: Wentia 15, 25 (1966)

4. Er zijn door Rosenberg onvoldoende argumenten aangevoerd voor zijn konklusie als zouden galactosyl-diglyceriden en chlorofyl molekulen in de membraan van chloroplasten een strukturele eenheid, te vergelijken met het sleutel-slot model, vormen.
A. Rosenberg: Biochem. Biophys. Res. Comm. 73(4), 972 (1976)

5. Bij een gezonde evenwichtige voeding van de zogende moeder is de toevoeging van extra vitamine D aan de voeding van het kind overbodig.
D.K. Lakdawala, E.M. Widdowson: Lancet I, 167 (1977)

6. Een bijdrage aan de vermindering van de kosten van de gezondheidszorg kan bereikt worden door de "therapie-trouw" van de patient te vergroten.
Ned. Tijdschrift v. Geneeskunde 121(24), 995 (1977)

7. De lokale fluoride-applikatie moet als een beter alternatief voor drinkwaterfluoridering worden beschouwd en dient sterk gestimuleerd te worden.

8. Er dient meer, maar ook minder met zwakzinnigen gespeeld te worden.

9. In vino sanitas.
Science 196, 1074 (1977)